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Replacement of marine-derived ingredients in meagre feeds (*Argyrosomus regius*): effects on growth performance and nutrient retention

João Filipe Baptista de Moura

Dissertação de Candidatura ao grau de
Mestre em Ciências do Mar e
Recursos Marinhos - Especialização em Aquacultura e Pescas
submetida ao Instituto de
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Abstract

In recent years we have witnessed a large increase in demand for fish meal and fish oil by the aquaculture industry while their production is relatively stable since the early '90s. Given the high rate of growth of aquaculture in a global level, it is urgent to find alternative sources for formulation of new diets in order to fight against the limited stocks of fishmeal and fish oils, which sooner or later will not be able to cover their demand. Vegetable proteins and vegetable oils have been used in recent years as a major alternative to the conventional methods of formulating diets based on marine resources. Although not representing a definitive solution to the supply feed needs of marine fish, they have been used with relative success in partial replacement of fishmeal and fish oils. The meagre (*Argyrosomus regius*, Asso, 1801), our fish model, is one of the largest sciaenid in the world. It is a species recently introduced in intensive aquaculture with great potential to fight for a place in the current market, competing with other species such as sea bass (*Dicentrarchus labrax*) or gilt-head sea bream (*Sparus aurata*). Being a carnivore species with a high protein requirement, it is with interest that studies are conducted on the impact of partial replacement diets with plant proteins and vegetal oils in their diet. 900 meagre juveniles were randomly assigned to 12 tanks (75 each) in order to obtain triplicates with four isoenergetic and isoproteic diets, only differing in the proportions of marine or plant sources in its constitution . They were fed *Ad libitum* twice a day and one more in the evening, by automatic feeders, for 3 months. In the end, five fish were collected from each tank to have their proximal composition analysed. Nutrient retention and growth performance were also evaluated. Results obtained were somewhat unexpected, since the juveniles have successfully tolerated the use of diets with significant percentages of substitution by plant sources, without major changes during the growth stage or even in their chemical composition.

Keywords: *Argyrosomus regius*, partial replacement diets, plant feedstuffs, growth performance, proximal compositon, nutrient retention

Resumo

Nos últimos anos tem se assistido a um grande aumento na procura de farinhas e óleos de peixe por parte da indústria de aquacultura enquanto que a sua produção se encontra relativamente estabilizada desde o inicio dos anos 90. Dado ao elevado ritmo de crescimento da aquacultura a nível global, é urgente encontrar fontes alternativas para formulação de novas dietas de modo a combater os stocks limitados de farinhas e óleos de peixe, que eventualmente, não conseguirão cobrir a sua procura. Proteínas de origem vegetal e óleos vegetais têm sido usados nos últimos anos como principais alternativas aos métodos convencionais de formulação de dietas baseadas em recursos marinhos. Apesar de não se apresentarem como uma solução definitiva para o requerimento de alimentação nos peixes marinhos, têm sido usadas com relativo sucesso na substituição parcial de farinhas e óleos de peixe. A corvina (*Argyrosomus regius*, Asso, 1801), o nosso peixe modelo, é um dos maiores sciaenídeos a nível mundial. É uma das espécies mais recentemente introduzidas a técnicas intensivas de aquacultura e com grande potencial de lutar por um lugar no actual mercado, concorrendo com outras espécies como a dourada ou o robalo. Sendo uma espécie carnívora e com altos requisitos proteicos é com interesse que se estuda o impacto de rações com substituição parcial de proteínas e óleos vegetais na sua dieta. 900 juvenis de corvina foram distribuídos aleatoriamente por 12 tanques (75 por cada) de forma a obter-se triplicados com quatro dietas isoproteicas e isoenegéticas, diferenciando apenas nas proporções de farinhas e óleos com fontes em recursos marinhos e vegetais na sua constituição. Foram alimentados *Ad libitum* duas vezes ao dia e uma terceira ao anoitecer por alimentadores automáticos durante 3 meses. No final, 5 peixes foram recolhidos de cada tanque para terem a sua composição proximal avaliada. A retenção de nutrientes e a performance de crescimento foram também determinadas. Obtiveram-se resultados algo inesperados, visto que os juvenis toleraram com sucesso o uso de rações com percentagens significativas de substituição por fontes vegetais, sem grande alteração durante a etapa de crescimento ou mesmo na sua composição química.

Palavras-chave: *Argyrosomus regius*, substituição parcial de dietas, fontes vegetais, performance de crescimento, composição proximal, retenção de nutrientes

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1. Introduction

1.1 The state of the aquaculture

Aquaculture industry continues to grow by leaps and bounds every year worldwide, which can now be compared with fish catches and captures used in human feed habits (FAO, 2012). In fact, about half of the fish for human consumption comes from aquaculture, something that surely would have been unthinkable a few decades ago to some experts, mainly due to the discrimination of fish produced in captivity by most of the population.

By the beginning of this century, forecasts about human demand for food fish were near the 110 million tonnes (Mtn) per year by 2010 (Francis *et al.*, 2001). Global fishmeal and oil production averaged 6.5 and 1.3 million metric tonnes (mmt), respectively (Hardy, 2010), over the past 20 years and more recent data confirms the high demand of fish meal and fish oils by the aquaculture industry, which has also increased steadily over the past 20 years from approximately 15% to 65% and 85% for fishmeal and oil respectively (Hardy, 2010; Tacon & Metian, 2008). Due the greater demand for marine based meals and oils, between mid-2005 and mid-2008, the prices of fishmeal and fish oil rose 50% and 130%, respectively (Naylor *et al.*, 2009). To maintain this growth rate, it is vital that the food supply for this industry also shares a similar evolution (Tacon & Metian, 2008). Unsurprisingly, despite the technological innovations that are being achieved throughout the years, aquaculture has not yet managed to totally disassociate from capture fisheries, which are essential for the provision of food for the many diverse cultures that are practiced today. This leads aquaculture to an underlying paradox: while it can be a possible solution, it can also be a contributing factor to the collapse of fisheries stocks worldwide (Naylor *et al.*, 2000). To fight against the inevitable insufficient marine supplies, efforts are being conducted in the attempt of finding new alternative sources of nutrient inputs, so that aquaculture industry can become more sustainable. In recent years, we could witness a lot of works that successfully proved the viability of terrestrial plant meals and seed oils to help replacing important portions of fishmeal and fish oils on a healthy and effective growth of several marine fish species (Dias *et al.*, 2009; Hernández *et al.*, 2007; Tibaldi *et al.*, 2006). Still, this is one of the most annoying problems that aquaculture must deal with because its production is still dependent upon the provision of fishmeal and fish oil based diets and supply of nutrient inputs.

In 2010, world aquaculture production has achieved another all-time high, at 60 million tonnes (FAO, 2012). The greatest advances in this area have occurred in developing countries in Asia, Africa or South America; Asia itself accounted for 89% of world aquaculture production by volume, with high contribution from China (more than 60% of world aquaculture). While Northern American aquaculture has stopped expanding recently, South American production has showed a strong and continuous growth, with huge contributions from Peru, Chile and Brazil (FAO, 2012). Europe increased its production of marine and brackish water from 55.6 percent in 1990 to 81.5 percent in 2010, mainly due to marine cage culture of Atlantic salmon and other species (FAO, 2012). In addition, three quarters of European aquaculture production consists of finfish, while mollusc production decreased over the past years (from 61 percent in 1980 to 26.2 percent in 2010), representing one quarter of total production.

One curious fact about the real state of aquaculture is that some countries (the majority in Africa and Asia) with similar natural conditions for aquaculture exhibit colossal differences in terms of development, turning them ineffective in contributing fish produced in aquaculture for its food supply at the national level (FAO, 2012).

1.2 Aquaculture in Portugal

Portugal has strong maritime and marine fishing traditions since long ago and mostly because of its 950 km long coastline. Fishing has always been an activity of great importance to the populations established along the coast, however it is now a high-risk activity given the increasing scarcity of natural marine resources.

In our country, aquaculture seems to have been stagnated over the last two decades, approximately with a total production of 8 thousand tonnes per year. It originally consisted of freshwater trout and bivalves bottom culture in tidal estuaries, but since 1990s marine species production showed an overall increase (FAO, 2010). The most important species produced in captivity are bivalves like grooved carpet shell, oysters and mussels. There is also a significant production of gilt-head sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*) from marine aquacultures and trout from freshwater units. Algarve is the region where aquaculture currently offers more jobs, about 2500 (Vivalgarve, 2010), being way higher than any other Portuguese region (FAO, 2010).

It is known that Portugal has excellent conditions for this practice, with special emphasis in the south region of the country, which in theory would make the country a main target for major investment in this area. Another fact that could be determinant for this sector's development is the amount of the of the total expenditure on fish products: Portugal along with Spain, France, Italy, Germany and UK account for 85% of total expenses in the European Union (FAO, 2010).

Nevertheless, Portuguese aquaculture is far from being at its best. Despite all ideas of promoting national production the truth is that aquaculture remains stagnant. National fisheries policy regarding aquaculture always aimed to increase production, product diversity and product quality in order to turn this sector in a competitive position (FAO, 2010). However these measures have turned out to be a total failure given that aquaculture remains with the same production as it had in late 80s. Several factors are responsible for the lack of development of Portuguese aquaculture but the main reason appointed for this problem is the difficulty in obtaining the permits necessary for the submission of new projects. A lot of time is required to study new projects submitted which leads possible investors to give up half-way before final approval. The low sale price of Greek and Spanish products on the markets as well as the current situation of the national economy does not help either. One curious fact about Portuguese aquaculturists is their refusal to do business with the national hypermarkets, because of their interest to buy at the lowest possible price (Vivalgarve, 2010). In contrast, hypermarkets and other big commercial facilities complain about the low dimension and the lack of capacity to provide large quantities of demanded products. Due to this crisis, several aquaculture companies have declared bankruptcy, such as “Viveiros Vila Nova SA”, in Vila Nova de Milfontes (2010), and Timar, in Tavira (2009). Those companies had a great impact on the total tonnes of fish produced in Portugal, leaving “Acuinova” as the leading company. In fact, this unit was presented as the largest unit of turbot farming in the world. However, in the last year an offshore area along the Algarve coast was finally cleared to allow the emergence of new businesses. It is expected that national production rises over the next year due this new opportunity to develop national aquaculture.

It is evident that Portugal’s aquaculture is way underrated and the need to turn this situation is crucial. There are enough reasons that are necessary for a sustainable development of aquaculture in this country; however Portuguese government and fisheries policy keep failing over the past years to finally launch this sector to its deserved success.

1.3 Aquaculture diversity

About 600 aquatic species are produced nowadays in captivity, using more or less sophisticated systems of fresh, brackish or marine waters (FAO, 2012). The main reason for this huge aquaculture development is undoubtedly the increased demand for different protein-rich food sources, as the world population increases at a high rate. The assessment of diversification of cultured species must aim at winning more important markets in order to ensure a large-scale development of aquaculture, focusing three main points: expansion of the market, the spreading of risk and the increasing of efficiency (Abellán E. & B., 1999).

New species introduced to aquaculture would certainly contribute to the expansion of the sector and may represent a solution to avoid currently saturated markets of commercialized fresh fish, like the most common gilt-head bream and sea bass. However there are some issues regarding newly introduced species, with high concern about alien species. These species may have environmental as well as social and economic impacts which should be studied in order to lower the risks and reap the maximum benefits, so that a plan for their responsible use may be developed and implemented (Bartley, 2013).

As the diversification of aquaculture increases, the probability of income fluctuations risks lowers and can be of value for a company that is able to produce more different marine finfish species than a competitor, as there is always some degree of diversification (Bartley, 2013). It can be also be a way to improve efficiency, since new species can be reared using the same type of technology on different seasons (Bartley, 2013). Production of different types of species can also reduce the impact of pathogenic agents on the company income, lowering the risk against serious diseases (Bartley, 2013). The culture of new species, as the one presented on this study - *Argyrosomus regius* - has been proposed as a basic mean to overcome saturated markets in Mediterranean aquaculture (Grigorakis *et al.*, 2011).

1.4 Meagre (*Argyrosomus regius*, Asso, 1801)

Argyrosomus regius, commonly known as meagre, is one of the largest sciaenid of the world reaching near 200 cm in total length and about 50 kg (Quèmèner, 2002), with a wide distribution that commonly occurs in temperate to tropical coastal waters and estuaries throughout the world (Sasaki, 1989), ranging from Norway to Senegal along the Atlantic coast and even including all the Mediterranean Sea (Chao, 1986) and Black Sea (Poli *et al.*, 2003). This species belongs to the *Sciaenidae* family, one of the largest perciform families in number of species and distribution, including approximately 78 genera and 287 species worldwide (Chao, 1986) and the vernacular english names of croakers and drums refer to the characteristic vocalization of the family (Sasaki, 1989) as they are able to produce sounds.

Being a semi-pelagic species, meagre can be easily found on coastal waters; big schools of meagre can even be found around wrecked ships that were sunk to create habitats for commercial species. It is know that meagre is an anadromous species, as they migrate to estuaries in the reproduction season at the end of May. It is by this time of year that is possible to hear males producing a typical deep sound, by pushing their abdominal muscles against the swim bladder. They remain on estuaries to spawn until July; after that they go back to feed along the coast and remain on shallow waters until winter season comes. By that time, meagre return to deeper waters in order to survive colder conditions, considering that growth is mainly achieved during summer. Juveniles remain on these nursing areas until summer ends, migrating 20-40 m to coastal to spend the winter. After that they return to estuaries, so they can feed on small demersal fish and crustaceans. Temperature is crucial at this time, as juveniles need around 20-21 ºC to feed. After they reach 30-40 cm they start to feed on pelagic fish and cephalopods.

Meagre has a relatively big head with elongated body (fusiform) with a mouth at the terminal position without barbils. Being a carnivorous species, meagre has large and strong teeth located on the outer zone of the jaw. Its body colour is silver-grey with bronze traits dorsally and has a lateral line that extends onto its caudal fin. Meagre's second dorsal fin is much longer than the first one and its anal fin has a first short spiny ray and a second very thin one. This species also has very large otoliths. One of the showiest characteristics of this species is the presence of bright spots along their lateral line.

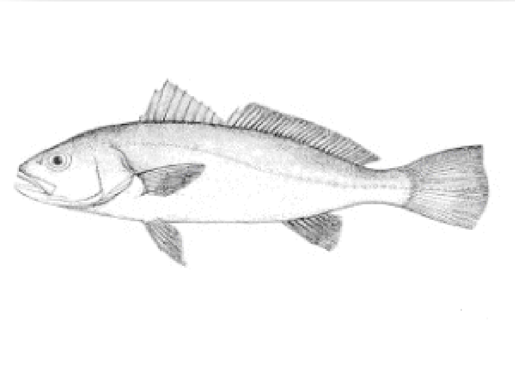

Fig. 1 - *Argyrosomus regius,* Asso 1801

Fig. 2 – Meagre's espacial distribution

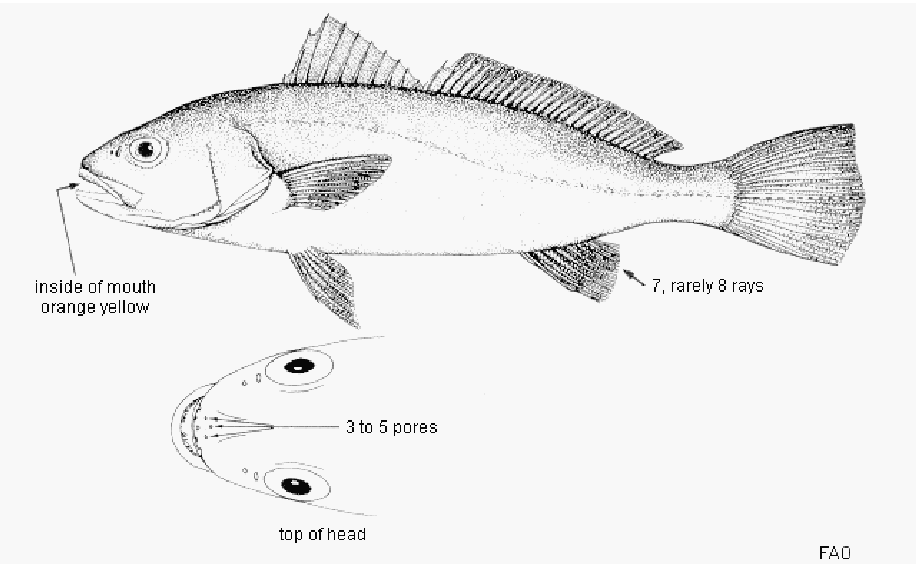

Fig. 3 – Morphological characteristics of meagre. Adapted from Monfort, 2010

Table 1 – Production of meagre from capture fisheries in tonnes (1980-2008) (Monfort 2010)

	1980	1990	2000	2001	2002	2003	2004	2005	2006	2007	2008
Total world[a]	3 207	5 261	4 108	4 046	5 926	6 340	8 941	9 337	8 250	4 840	5 724
Egypt	269	113	776	1 038	1 372	1 414	2 411	1 232	2 107	1 602	1 202
France		179	189	162	156	101	525	1 263	1 356	1 204	13
Ghana										233	2 042
Guinea–Bissau			394	372	730	337	310	277	260	240	240
Israel	10	67	288	223	273	249	144	6	2	22	22
Mauritania		2 000	600	600	600	950	1 200	1 500	900	1 320	1 230
Morocco*	1 160	2 544	1 755	1 534	2 047	3 102	4 160	4 722	3 387	**	**
Portugal	937		4	6	36	40	46	172	154	143	159
Spain	816	94									
Turkey	15	193	70	50	63	75	62	96		60	56

Source: FAO (2010a)
** data from FAO (2008). ** In FAO (2010a), 2007 and 2008 meagre were grouped with all Sciaenidae.*
[a] total world production reflects the fact that in some years and countries meagre was grouped with all Sciaenidae.

Global fisheries production ranges from 5 000 to 10 000 tonnes per annum, while the production of meagre from capture fisheries in Europe (geographical perimeter) is low ranging from a few hundred tonnes to 1 500 tonnes in the different countries (Monfort, 2010) (Table 1).

Table 2 - Production of meagre from aquaculture in tonnes (1997-2008) (Monfort 2010)

	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
France	30	30	30	33	35	165	100	147	267	282	282	300
Italy						131		696	186	172	192	300
Spain							3	16	347	489	251	1 374
Portugal									47	47	25	15
Greece												*240
Turkey												**512
Malta										#28	#12	#12
Egypt												2 031

*Source: FAO (2010b), * Barazi (2010, pers. comm), ** Deniz (2009), # Vassallo Agius (2010, pers. comm.)*

Meagre culture started in the late ‘90s, being relatively a new species in the aquaculture industry. First cultures were done by French and Italian aquacultures and it had the first commercial production in 1997 in France (Monfort, 2010). In the following years meagre’s culture spread to other Mediterranean countries, like Spain, Greece and even Portugal and, unless some exceptions, its production is rapidly increasing (Table 2). Nowadays, more than half of meagre’s production will be produced in Spain and by 2010 it was expected that European production would reach approximately 10 000 tonnes (Monfort, 2010). In our country, the fish is well known and most appreciated in the south of the country (Algarve) and is commercialized with a large size (over 5 kg) (Monfort, 2010). Flesh quality is considered exceptional and highly nutritious, and the species name “regius” (i.e., royal) was given because of its highly esteemed flesh quality (Papadakis *et al.*, 2013; Poli *et al.*, 2003).

1.5 Meagre aquaculture

In fact, meagre's aquaculture is quite recent: 1997 is the first year with records of production of this fish, in France and Italy. Nowadays meagre's aquaculture is spread to other Mediterranean countries, and production is rapidly increasing, with special emphasis in Spain, that in 2008 had a total production of 1 374 tonnes (Monfort, 2010).

Its market price is around 6 euros/kg, which can be considered an important factor to keep expanding the bonds of meagre aquaculture. This makes meagre a real opponent to sea bass or gilt-head sea bream, as its price per kilo is much more valuable comparing to these species. Considering its biology, that allow meagre to be resistant to changes in temperature and salinity, as well as its high fertility, its fast growth, its good chemical composition of its fillet (Piccolo *et al.*, 2008) and good consumer acceptance this fish aquaculture has a lot of reasons to succeed in the Mediterranean region.

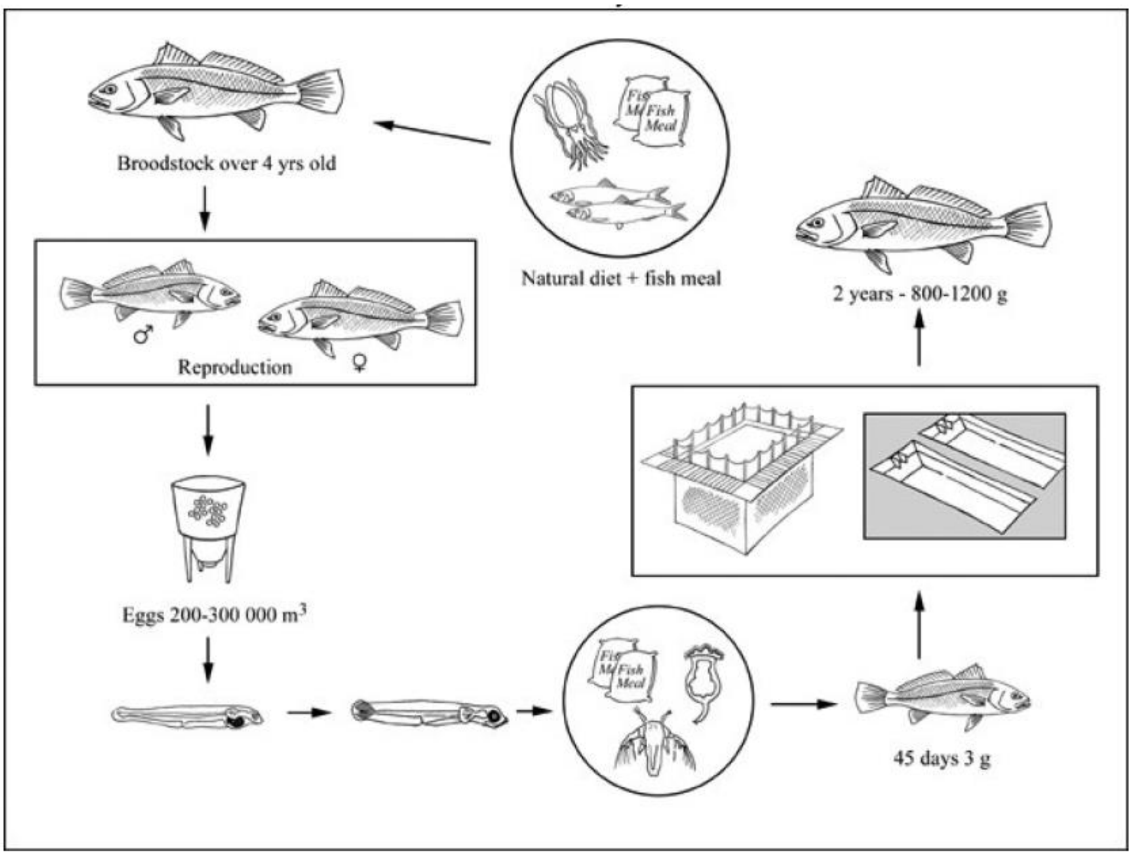

Fig. 4 – Meagre's aquaculture cycle. Adapted from Monfort, 2010

Techniques applied nowadays on the cultivation process of meagre are similar to those of the sea bass and gilt-head sea bream. Despite natural meagre reproduction taking place between spring and summer with several postures, it is usual that hormonal treatments are needed to secure the captivity reproduction of this species (Cárdenas, 2010). Larvae survival is also good, with survival around 15% to 40% 30 days after hatching and 15% 60 days after hatching (Cárdenas, 2010). A recent work proved the ability of meagre larva to ingest inert diet from a very early age, without negative impacts on digestive physiology and fatty acid compositions, which decreases the use of live feeds and enhance growth (Pousão-Ferreira *et al.*, 2013). As mentioned before, meagre younglings have a huge growth, mainly at the larval stages, beating reared gilthead sea bream for example. Being an eurythermic and euryhaline species, it shows high growth rate during summer, when water temperatures are around 21 ºC (Le François *et al.*, 2010). Reared meagre behaviour during the pre-fattening and fattening stages is also decent, either in tanks or cages, suggesting that this fish does not display high stress levels when in captivity, when in similar conditions of other reared marine species. As a proof of this, reared meagre's mortality is so low that could even be absent during its growth. Furthermore, it was not still pointed a serious disease or menace that could turn its production to be a total failure.

Fig. 5 – Reared juvenile of this experiment

1.6 Fish nutrition of meagre

Little information exists about the dietary requirements for lipids and proteins that can be used for the formulation of specialized diets for this species. Meagre is a carnivorous species and in the wild it feeds on Mysidacea, Decapoda and Teleostei (Chatzifotis *et al.*, 2012). As the majority of carnivorous fish, meagre has a relatively short digestive tract, which represents 70% of its total body length. Despite that, this fish is still able to ingest large prey (Cárdenas, 2010). Nowadays, this species is feed on pelleted diets and is currently raised on feeds used for sea bass (*Dicentrarchus labrax*) and sea bream (*Sparus aurata*), as there are no optimized diets for meagre yet. Despite the low information that exists as to the requirements for proteins and lipids, it is known that meagre, as being a carnivorous fish, requires greater quantities of protein to properly grow. As for lipids in general, marine species requires longer-chain n-3 and n-6 polyunsaturated fatty acids (PUFA) for optimal growth and health, since they do not show rates for bioconversion of C18 PUFA into C20 and C22 HUFA that would allow n-3 HUFA requirements to be met (Benedito-Palos *et al.*, 2008). Meagre is not different regarding this requirement of essential fatty acids (EFA). The increase in dietary lipid level improves growth, feed and protein efficiency, as it spares proteins that could otherwise have been catabolized and used as an energy source (Chatzifotis *et al.*, 2012). This can be proved by salmonids, the atlantic halibut or the dentex, which are fishes reared with diets with high percentage of fat (Helland & Grisdale-Helland, 1998; Hillestad & Johnsen, 1994; Skalli *et al.*, 2004). However there are some cases where this is not verified, mainly on fishes like the gilt-head sea bream and the sea bass. It would also seems that meagre does not benefit from diets that contain more than 17% of fat (Chatzifotis *et al.*, 2012). Besides that, it appears that elevated dietary lipids may result in excessive fat deposition in the visceral cavity and tissues of the meagre, as it happens also in the sea bream or the rainbow trout (Weatherup *et al.*, 1997). All these works suggests that diets made specifically for meagre should have at least around 50% protein and 17% lipids. This way, meagre is a fish that provides low-fat meat even under intensive farming conditions (Piccolo *et al.*, 2008).

1.7 Sustainability of aquafeeds

In 2010, 20.2 million tonnes (representing about 14% of total fish production) was destined to non-food purposes, of which 75 percent (15 million tonnes) was reduced to fishmeal and fish oil (FAO, 2012). One real fact is that the demand for fishmeal production is growing at a very fast rate, mostly because of asian aquafeed industries, turning it more and more expensive every day. On the another hand, world production of grains and oilseeds has increased over the past two decades as a result of higher yields and increased plantings (Hardy, 2010). It is already recognized that the utilization of plant feedstuffs is essential for future development of aquaculture so that cheaper sources of protein have been investigated over the past years in order to identify economically viable and environmentally friendly alternatives to fish meal and fish oil (Gatlin *et al.*, 2007). Research is now focused on commodities such as oilseeds (especially soybeans), meat byproducts (such as blood meal, bone meal or feathers) and microbial proteins (Naylor *et al.*, 2000). Unfortunately one big problem of using this kind of alternative meals is that vegetable proteins have inappropriate amino-acid balance and poor protein digestibility (Francis *et al.*, 2001; Naylor *et al.*, 2000), leading to slower than normal growth rates. A number of negative effects of plant-based ingredients have been identified last years, such as hindgut inflammation, reduced appetite or protease inhibition have been recognised, especially when plant-based proteins are used in a majority over animal proteins (Brinker & Reiter, 2011; Francis *et al.*, 2001). This is absolutely one of the main challenges that the scientific community is dealing with in the field of aquaculture in which balance between advantages and constrains is crucial.

Nowadays research in fish meal replacements is a main focus of the science community, however little is known about their real impact on meagre. Fish meal has traditionally been a major ingredient in fish feeds because of its protein quality and palatability (Boonyaratpalin *et al.*, 1998), especially for carnivorous fishes, in contrast to meals with different protein and lipid sources. Evaluating the potential of an ingredient as a sole protein source, besides conventional nutritional criteria (indispensable amino acid profile, nutrient bioavailability, digestible energy supply), the adverse effects of anti-nutritional factors (ANF) should also be taken into consideration (Kaushik *et al.*, 1995). Among plant proteins, soybean meal (SBM) is the most promising candidate for partial or total replacement of fish meal in fish diets (Boonyaratpalin *et al.*, 1998). Actually, the main problems of vegetarian meals are those ANFs, contributing to a low digestible efficiency and even causing major problems on the digestive system of carnivorous fishes. Different ANFs have been identified in soybeans, like protease inhibitors, lectins, antigenic proteins, phenolic compounds or even oligosaccharides (Kaushik *et al.*, 1995). These compounds are easily found on plants, as they take part on their defensive mechanism against predators like insects and consequently vegetal based meals are also rich on this kind of substances. Phytates or phytic acid is also considered an antinutritional factor in vegetable meals, as most oilseeds and cereals contain 1-2% of it (Vielma *et al.*, 2002). Phytate, a cyclic inositol compound containing six phosphate groups, mainly serves as a phosphorus (P) store in plants and represents approximately 50% of the P in oilseeds. It is able to chelate di- and trivalent minerals, decreasing their availability to animals and also nonselectively binds to proteins and has been shown to inhibit enzymes including pepsin, trypsin and alpha-amylase (Vielma *et al.*, 2002). The most important ANFs and where they can be found are shown below, on Table 3.

In an attempt to reduce those problems, aquaculture plant derived meals are mixed with fish meal based meals, in order balanced them out, so the quality of fish flesh is ensured. Furthermore, there are some papers proving that heat treatment of soybeans can improve growth performance in some fish, like the trout (Sandholm *et al.*, 1976), the common carp or even the coho salmon (Arndt *et al.*, 1999). However it is also know that physical heating can be the cause of losing essential amino acids (Elangovan & Shim, 2000; Plakas *et al.*, 1985); therefore this treatment may be considered as a double-edged sword, as it destroys some of the ANFs, but in another hand impoverishes the nutritional content of the soybean. There are also other specific treatments that are required to remove other ANFs, like sieving, chemical extraction and biological enzymatic treatments processes. For example, phytate which is relatively heat-stable cannot be effectively removed without enzymatic reactions (Vielma *et al.*, 2000).

As for studies that have been undertaken to evaluate the organoleptic quality of the flesh affected by partial or total replacement of fish meal in fish diets, there are some reports that show us that flesh quality was not affected by it, like rainbow trout or yellowtail (Kaushik *et al.*, 1995). On the other hand, it has been reported that the Siberian sturgeon *Acipenser baeri* had a significant estrogenic action resulting in increased concentrations of circulating vitellogenin (Pelissero *et al.*, 1991). In marine fish like the meagre very little is known about the organoleptic quality of the flesh on fish reared by partial replacement fish meals, however in similarly reared species like the sea bass or the gilt-head sea bream there are no records of drops in flesh quality.

Table 3 – Plant derived feedstuffs and their antinutritional factors

Plant-derived nutrient source	Antinutrients present
Soybean meal	Protease inhibitors, lectins, phytic acid, saponins, phytoestrogens, antivitamins, allergens
Rapeseed meal	Protease inhibitors, glucosinolates, phytic acid, tannins
Lupin seed meal	Protease inhibitors, saponins, phytoestrogens, alkaloids
Pea seed meal	Protease inhibitors, lectins, tannins, cyanogens, phytic acid, saponins, antivitamins
Sunflower oil cake	Protease inhibitors, saponins, arginase inhibitor
Cottonseed meal	Phytic acid, phytoestrogens, gossypol, antivitamins, cyclopropenoic acid
Leucaena leaf meal	Mimosine
Alfalfa leaf meal	Protease inhibitors, saponins, phytoestrogens, antivitamins
Mustard oil cake	Glucosinolates, tannins
Sesame meal	Phytic acid, protease inhibitors

Data extracted from (Francis *et al.*, 2001)

2. Objectives

Meagre (*A. regius*) increasingly appears to be the new species with the most potential to face other reared marine fish like the sea bass and sea bream. In the last years a lot of research has been focused on improving meagre's farming efficiency so this species can finally earn a place on the market. This study aims to determine the growth performance, proximal composition and nutrient retention values of the meagre reared during 14 weeks and using 4 types of partial replacement diets with different sources (marine and vegetal), all of them containing the same amount of proteins and fat, only varying in the percentage of the protein and oil source. Chemical proprieties of the water were kept constant all along the experiment, except for the temperature, which was impossible to maintain constant values.

3. Materials and Methods

3.1 Diets

The experimental diets used on this work were formulated by SPAROS Lda, with the purpose of studying the impact of isoenergetic and isoprotein diets (Table 4 and 5), but different in the percentages of sources of protein and oils in our fish model: the meagre, *Argyrosomus regius*. So we have a control diet FishMeal/FishOil (FMFO), with most of protein coming from fish meal and only fish oil; a FishMeal/VegetalOil (FMVO) with most protein coming also from fish meal and most oil coming from vegetal sources; a PlantProtein/FishOil (PPFO), where we have a high percentage of plant protein and only fish oil; and a PlantProtein/PlantOil (PPVO), with high percentage of vegetal protein and high percentage of vegetal oil. Our control diet, FMFO, was formulated based on balanced commercial diets used for gilthead seabream, with protein levels of around 50% and about 17% of lipids, as until now there is no occurrence of diets designed specifically for meagre. This control diet is composed of ingredients commonly available on the actual market, such as fishmeal, concentrate of fish proteins, squid meal, soy protein concentrate, corn and wheat gluten as well as whole corn and whole wheat, fish oil, vitamins and some specific amino acids (to compensate for the loss of some vital amino acids, required for a healthy growth of the fish).

Following this, diets with high percentage of vegetal protein (PPFO and PPVO) were formulated with the same levels of protein, with the difference on their percentage of protein source, which had their fishmeal source reduced while replacing it by vegetal ingredients. Differing to the control, these diets were composed using a heavier amount of soy protein concentrate as well as of corn and wheat gluten. Pea protein concentrate was also added, so the percentage of 50% of protein was reached. In the other hand, diets with vegetal oil (FMVO and PPVO) were composed by rapeseed oil, linseed oil and palm oil. Diet formulations are shown in Table 6.

FMFO, FMVO, PPFO and PPVO will be analysed together, through a two way analysis of variance, as they have the same two factors varying between them (the protein source and the oil source).

Table 4 – Abbreviations of the diets

Abbreviation	
FMFO	FishMeal FishOil
FMVO	FishMeal VegetalOil
PPFO	PlantProtein FishOil
PPVO	PlantProtein VegetalOil

Table 5 – Composition of experimental diets

Diet	**DM %**	**Prot %**	**Fat %**	**Ener KJ/g**	**Pho %**
FMFO	94,79	51,20	17,68	22,90	1,38
FMVO	95,05	50,52	18,10	22,91	1,36
PPFO	94,79	51,29	17,40	23,13	1,32
PPVO	94,99	51,27	17,12	22,70	1,32

Table 6 – Formulation of experimental diets

	FMFO	FMVO	PPFO	PPVO
	%	%	%	%
Fishmeal 70 LT	25,00	25,00	10,00	10,00
Fishmeal 65	15,00	15,00	7,50	7,50
Fish solubles protein concentrate (CPSP 90)	2,50	2,50	2,50	2,50
Squid meal	2,50	2,50	2,50	2,50
Chicken liver hydrolisate				
Feathermeal hydrolisate				
Haemoblobin powder				
Soy protein concentrate (Soycomil PC)	5,50	5,50	8,00	8,00
Pea protein concentrate (Lysamine GP)			4,50	4,50
Wheat gluten	5,00	5,00	14,00	14,00
Corn gluten	7,50	7,50	10,00	10,00
Dehulled solvent extracted soybean meal	5,00	5,00	5,00	5,00
Whole wheat	4,00	4,00	4,00	4,00
Whole peas	10,00	10,00	9,00	9,00
Fish oil	13,00	5,20	14,50	5,80
Rapeseed oil		1,30		1,45
Linseed oil		3,90		4,35
Palm oil		2,60		2,90
Poultry fat				
Vit & Min Premix	1,00	1,00	1,00	1,00
Betaine	0,30	0,30	0,30	0,30
Soy lecithin	0,50	0,50	0,50	0,50
Binder (Kieselghur)	0,50	0,50	0,50	0,50
Anti-OX (Paramega PX Dry)	0,20	0,20	0,20	0,20
Mono calcium phosphate			2,50	2,50
Glycerol	2,50	2,50	2,50	2,50
L-Lysine			0,80	0,80
DL-Methionine			0,20	0,20
TOTAL	100,00	100,00	100,00	100,00

3.2 Rearing system

This experiment was conducted over 14 weeks in the Olhão Aquaculture Research Station (or “Estação Piloto de Piscicultura de Olhão - EPPO), between September and December of 2012. Twelve fiberglass tanks of 1500 l were used to hold 75 meagre juveniles each, in a total of 900 fishes. All those fishes started with a weight of 55 g and 125 days of life and were placed randomly in each tank after manually screened. The density of fishes per tank was no higher than 2,75 kg/m^3. The tanks were organized so it was possible to make triplicates concerning the four diets, which makes three tanks for each diet. They were kept in an open system of water circulation, which was pumped from the water reserve in Ria Formosa after being filtered and treated and were subjected to a photoperiod of about 15 h on daylight. Water parameters were recorded every day, with high concern about the water temperature and salinity, so that equality was assured between tanks. It was necessary to use a boiler to reduce water temperature variation along the experiment, as in the season this experiment went on water temperature drastically drops. With the boiler working it was assured that the fishes kept on feeding, as it was essential for the experiment.

Meagre’s juveniles were manually fed two times per day, one in the middle of the morning and another in the afternoon (10:30 h and 15:30 h, respectively). They were also fed using an automatic feeder, which worked at 21:30 h, so that juveniles could have a last meal to prevent cannibalism between them, especially at the early phases of the experiment. Concerning the manual feeding of juveniles, it was done in a patient way as meagre is not a voracious fish like the gilt head sea bream. In half an hour, all fish were considered to be well fed, as far as they do not show any signs of hunger or other strange behaviour. At the end of every meal, all excess food deposited in the bottom of the tank was collected and then weighed. To remove water weight from the wet diets after collecting it from the tanks, it was necessary to calculate a factor that represents how much weight a dry diet gains after being on 2l of water at 18º C for two hours. For each treatment we calculated its factor (Table 7), starting with 10g of dry diet and then dividing its wet weight by its dry weigh.

Table 7 – Wet weight factor of experimental diets

Treatments	Factor
FMVO	2,2
PPVO	2,3
PPFO	2,3
FMFO	1,9

Fig. 6 – One of the 12 fiberglass tanks used in the growth trial

3.3 Samplings

During this experiment, five samplings of meagre's juveniles were done: one at the beginning, three intermediary samplings and one at the end of the experiment. In the first sampling all fishes were individually weighted and ten of them were collected and frozen, for future proximal analysis of the carcass, to evaluate the quantity of proteins, lipids, ashes, dry matter, phosphorus and energy. In all intermediary samplings, juveniles were weighted in groups of 10 fishes. In the final sampling, all fishes were weighted and measured individually. Also, five juveniles with apparent mean size and weight of each tank were collected and frozen, for posterior proximal analysis.

Fig. 7 – Sampling location

3.4 Analytical methods

3.4.1 Zootechny data

All data referring to the growth performance of meagres under the treatment of the various diets was assessed via the final body weight (FBW), specific growth rate (SGR), feed conversion ratio (FCR), voluntary feed intake (VFI) and protein efficiency ratio (PER). All fish were weighted one by one at the end of the experiment to get the value of the average FBW. SGR, FCR, VFI and PER were evaluated based on the following formulas:

$$\text{Specific growth rate (SGR)} = \frac{100\ (\ln \text{FBW} - \ln \text{IBW})}{\text{Nº of days}}$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Total dry feed intake (g)}}{\text{Wet weight gain (g)}}$$

$$\text{Voluntary feed intake (VFI)} = \frac{\text{Total dry feed intake (g)}}{\frac{\frac{\text{Initial Biomass + Final Biomass (g)}}{2}}{\text{Nº of days}}} \times 100$$

$$\text{Protein efficiency ratio (PER)} = \frac{\text{Wet weight gain (g)}}{\text{Protein fed (g)}}$$

3.4.2 Whole body composition

After the rearing experiment, fish carcasses were crushed at the SPAROS facilities, using a shredder machine. After that, crushed fish were freeze dried so they could be analysed. Proximal composition of the whole fish carcasses was made using the laboratory facilities and equipment of LANUCE, in CIIMAR. All proximal analyses of the carcasses were performed in duplicate.

Dry matter percentage of the carcasses was calculated using the official method by the Association of Official Agricultural Chemists (AOAC) in which they were dried at 105º C for 24 h in an Binder oven, while ash content of the fish carcasses was calculated from the weight change before and after incineration of approximately 24 h in a *Nabertherm* muffle at 500 ° C, until obtaining constant weight (method also by AOAC).

Crude protein was obtained using a LECO FP-528 combustion nitrogen determinator. To get the determination of total nitrogen in the form of N_2 this machine burns a small amount of sample in pure oxygen at high temperatures, around 900 ºC. The overall N_2 is measured by a thermal conductivity cell controlled by a microprocessor and then converted to equivalent protein.

Fat was determined using the Soxtherm official method also by the AOAC. The principle of this method consists in the quantification of total lipids in a sample by the extraction using an organic solvent, in this case, petroleum ether. A Soxtec 2500 machine was used to reproduce this method, as well as a Binder oven.

Gross energy was obtained with the help of an adiabatic bomb calorimeter IKA C2000, IKA-Werke GMBH & CO.KG, Staufen, Germany. The principle of this machine consists in determining gross energy of organic matter, using the combustion action of oxygen, in a decomposition pot at pre-set conditions. A precision Beckmann thermometer measures the temperature before and after the combustion, causing the water temperature to rise first, and then gradually returns to the ambient temperature, to a constant value. The variation in the temperature ΔT observed in the calorimeter is proportional to the heat liberated by the reaction and using this value it is possible to measure directly the amount of energy depending on the gross weight of the sample.

Finally, total phosphorus quantity was determined by the AFNOR V 04-406 method, with small modifications adapted due to the type of the samples and laboratory materials available in LANUCE. After digestion, in a Kjeldatherm Block unit, and wet oxidation at the temperature of 230 ° C with sulphuric acid and hydrogen peroxide, this method proceeds to the determination of orthophosphate by spectrophotometry molecular absorption at 820 nm.

3.4.3 Retention

Nutrient intake percentages or retention were also determined after the experiment. With this data we intended to understand how the fish reacted to the different treatments applied in terms of their capacity of storage of the different nutrients and energy given by the diets. Retention was calculated based on the following formulas:

$$\text{Retention nutrient (\% intake)} = \frac{\text{Total dry nutrient gain (g)}}{\text{Total dry nutrient intake (g)}}$$

$$\text{Retention energy (\% intake)} = \frac{\text{Total energy gain (KJ)}}{\text{Total energy intake (KJ)}}$$

3.5 Statistical analysis

To test the differences between the four main dietary treatments a two-way ANOVA was used, considering the protein and lipid source as variables (FMVO, FMFO, PPFO, PPVO). As routine for this kind of data analysis, all data was previously checked for normal distribution and homogeneity of variances. Data represented by percentages were arcsin transformed so they could be analysed. As usual statistical significance was tested at 0.05 probability level and all tests were performed using the SPSS v20 package.

4. Results

4.1 Growth experiment

The growth experiment had a duration of 102 days, starting the 15th September and ending the 11th December. During this period, water temperature suffered some variation, with the lowest recorded value of 15,6 º C in mid-November and the highest of 24,0 ºC in early-September. Mean temperature for the growth experiment was 19,53±2,29 ºC and despite all those variations in temperature between the end of summer and the beginning of autumn, all fish kept on feeding, which was essential for a successful trial. Figure 8 displays all water temperature variation in this growth experiment.

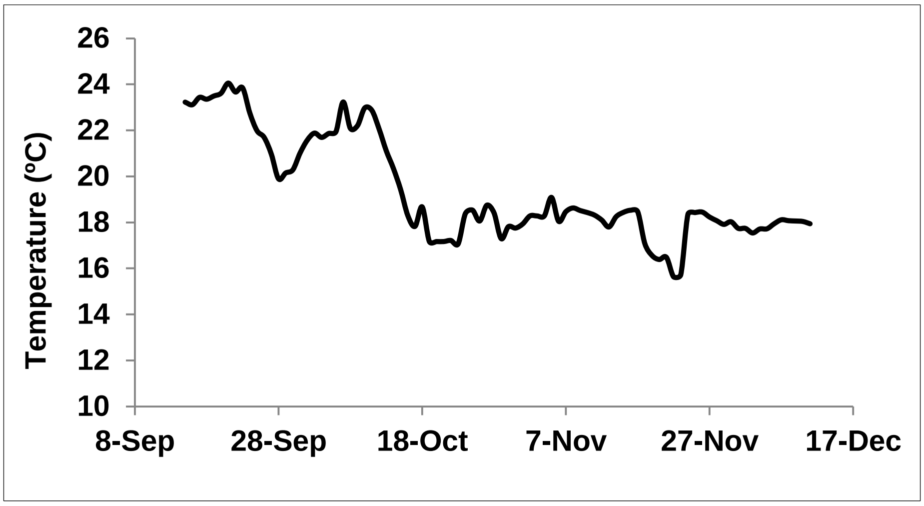

Fig. 8 - Water temperature variation

Development of the fish mean weight can be seen in Figure 9. The four main treatments to analyse had showed similar weight developments along the trial. Nevertheless, mean weigh of fishes treated with the PPFO diet was always higher than any other diet. FMFO and FMVO treatments had almost the same development, recording a similar performance along the experiment. PPVO treatment had similar results on the fish body weight until mid-October, and showed a slight drop over the next months.

Mortality could be neglected, considering that it was very low and that most fishes died by jumping out of the tanks (Table 9). Data referring to the various samplings that were made over this experiment can be checked in Table 8.

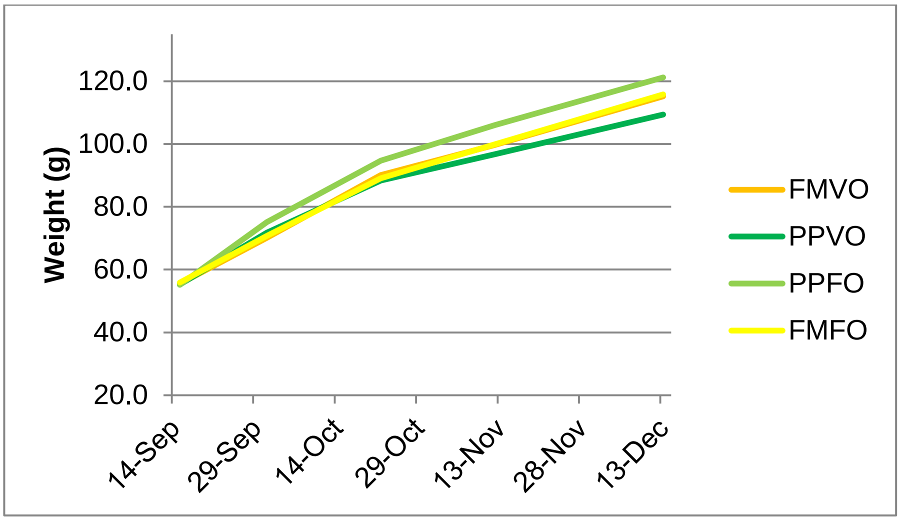

Fig. 9 – Fish mean weight over the experiment

Table 8 – Samplings data over the experiment (g)

Treatmets	15 - Sept	01 - Oct	22 - Oct	12 - Nov	13 - Dec
FMFO	55,8	70,7	89,1	99,9	115,8
FMVO	55,5	70,1	90,1	99,7	115,2
PPFO	55,1	75,2	94,7	106,0	121,2
PPVO	55,2	71,8	88,4	96,7	109,4

Table 9 – Mortality of juveniles

Treatmets	End (g)	Mortality %
FMFO	209	0,07
FMVO	220	0,02
PPFO	222	0,01
PPVO	215	0,04
Total	866	0,04

4.2 Zootechny data: growth performance

All data concerning the growth performance can be seen in Table 10. The final weight of fish bodies were fairly similar in all four main diets, ranging from fishes with a minimum mean weight of 109,5±3,9 g in the PPVO treatment to the maximum mean weight of 121,2±2,4 g of the PPFO treatment. There were almost no differences between the other two treatments, with average weights of 115,9±4,8 g and 115,2+7,8 g of the FMFO and FMVO, respectively. Average body weight of the fishes has doubled, approximately, since all treatments started with an initial body weight of 55 g. Final body weight was not significantly affected by the protein and/or the lipid source (Table 11).

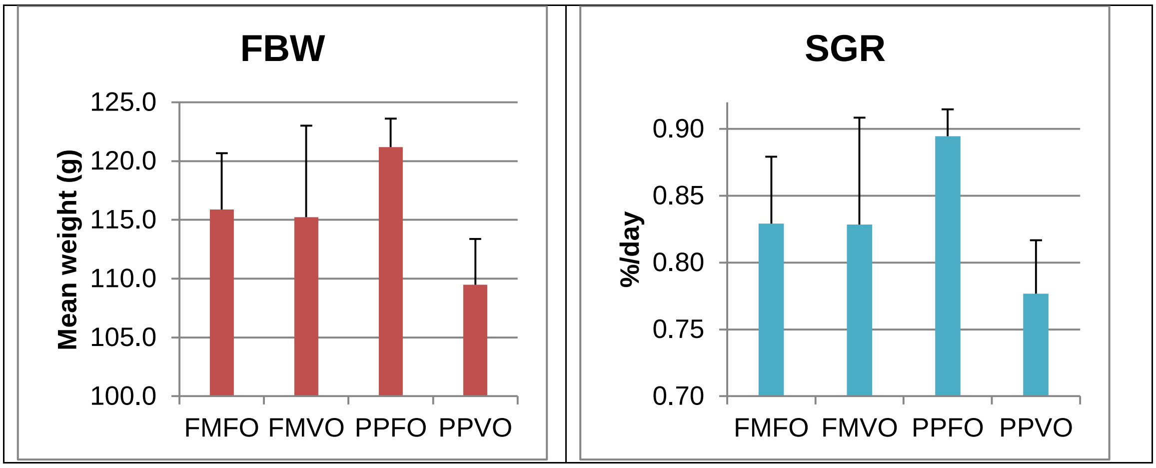

Fig. 10 – Final body weight and specific growth rate graphics of the dietary treatments

The higher value of the specific growth ratio index was achieved by the PPFO treatment with 0,89±0,02 %/day. FMFO and FMVO had almost the same average growth of 0,83±0,05 %/day and 0,83±0,08 %/day, respectively, while PPVO had the lower percentage of fish growth along the experiment with a mean of 0,78±0,03 %/day. These values are directly proportional to the final body weight; however, it appears that the protein and the lipid source do not affect the daily growth of meagre, as there is no significance as shown in Table 11.

Feed conversion ratio was affected by the interaction protein and the lipid source as well as only by the protein source of the diets (P<0,05) (Table 11). The best value was achieved by the PPFO treatment, with an index of 0,99±0,04. The other three treatments had also low values: 1,19±0,06; 1,16±0,10 and 1,16±0,04 for FMFO, FMVO and PPVO, respectively.

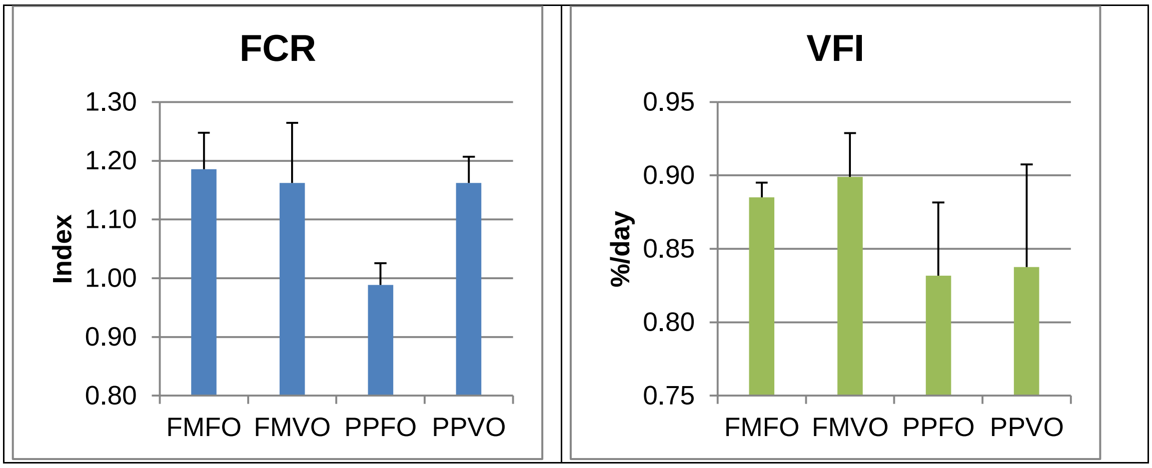

Fig. 11 – Feed conversion ratio and voluntary feed intake graphics of the dietary treatments

There were no significant results on the voluntary feed intake, as it was not affected by the protein or/and lipid source (Table 11). Following the results of the feed conversation ratio, in which PPFO obtained the best value, VFI values of these diets were in agreement with the previous indexes. The lowest value of VFI obtained was 0,83±0,05 %/day, with the PPFO diet, followed by 0,84±0,07 %/day with PPVO, 0,89±0,01 %/day with FMFO and 0,90±0,03 %/day with FMVO.

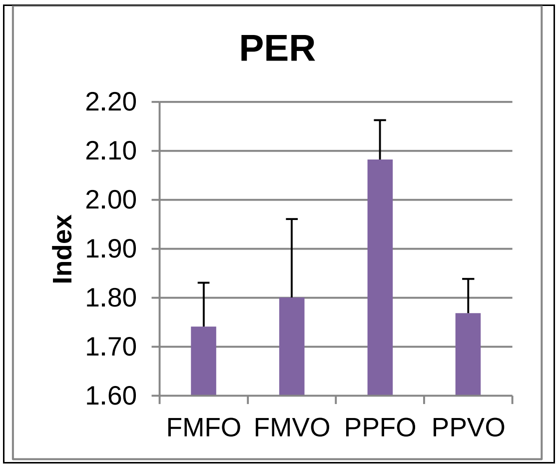

Fig. 12 – Protein efficiency ratio graphics of the dietary treatments

Analysing protein efficiency ratio we observed that PPFO treatment was way higher than any other dietary treatment, with an index of 2,08±0,08. All the others diets had similar results: 1,74±0,09 by FMFO; 1,77±0,07 by PPVO and 1,80±0,26 by FMVO. Statistical analysis also proves that PER was affected by the protein source as well as by the interaction between the two sources of protein and fat (P<0,05) (Table 11).

Table 10 – Growth performance of the various experimental diets (means and standart deviations)

Diet	IBW (g)	FBW (g)	SGR (%)	FCR	VFI (%)	PER
FMFO	55,8	115,9	0,83	1,19	0,89	1,74
	0,2	*4,8*	*0,05*	*0,06*	*0,01*	*0,09*
FMVO	55,5	115,2	0,83	1,16	0,90	1,80
	0,1	*7,8*	*0,08*	*0,10*	*0,03*	*0,16*
PPFO	55,1	121,2	0,89	0,99	0,83	2,08
	0,4	*2,4*	*0,02*	*0,04*	*0,05*	*0,08*
PPVO	55,2	109,5	0,78	1,16	0,84	1,77
	0,1	*3,9*	*0,04*	*0,04*	*0,07*	*0,07*

Table 11 – Statistical analysis of the growth performance

	P Value	P Value	P Value
	Protein Source	Lipid Source	Interaction
FBW	0,952	0,071	0,099
SGR	1	0,282	0,282
FCR	0,03*	0,087	0,034*
VFI	0,195	1	1
PER	0,033*	0,071	0,014*

Values with * are statistically different (P <0.05)

4.3 Whole body composition

Composition of body carcasses of meagre was also analysed after the growth experiment. Despite small differences among the treatments, all of them shared very similar percentages, as can be seen in Figure 13. Data about proximal composition can be checked in Table 12. In addition we also conclude the final body composition of the fish did not differ too much from their initial body composition.

Dry matter percentages had a very low variation amongst treatments, ranging from 27,56±0,30 (FMFO) to 28,87±0,40 (PPFO). After statistical analysis, it was observed that dry matter was affected by the protein source and also by the interaction between lipid and protein source ($P<0{,}05$) (Table 13).

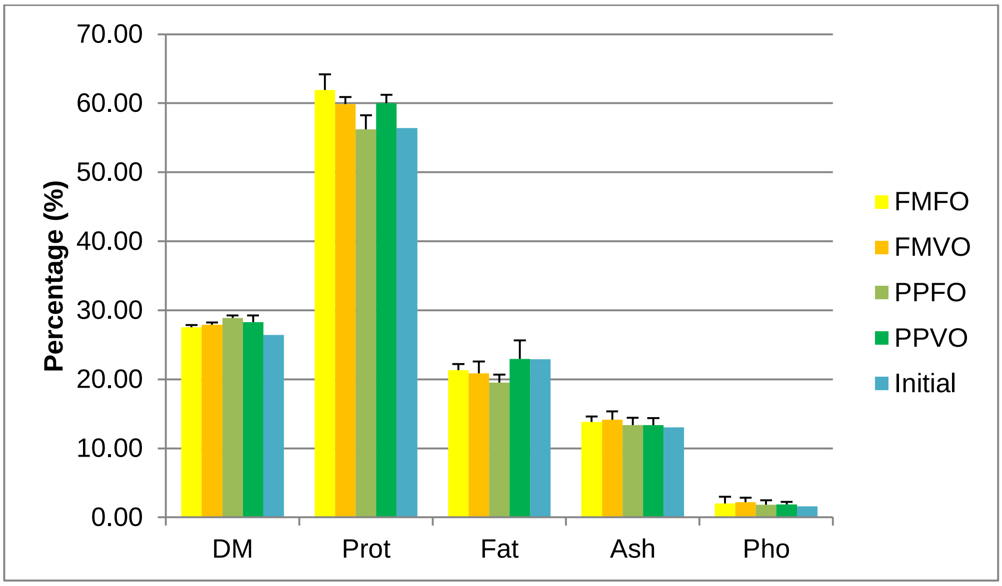

Fig. 13 – Whole body composition percentages of the various treatments

Protein percentages of these diets were all around 60%, with small deviation errors in each treatment. All percentages were higher than the initial carcasses of the fish, except for the PPFO diet, which was slightly smaller. The highest percentage of protein was observed for the FMFO treatment, with 61,90±2,30 %, while the lowest was 56,21±2,03 % by the PPFO diet. This work suggests that protein percentages of diets are significantly affected by the protein source of the treatments, as well as the interaction between protein and lipid source ($P<0,05$) (Table 13). Meagre's body percentage of fat was around 20% in all treatments and slightly lower than the initial composition of the fishes. Fat percentage varied between 19,52±1,15 %, by PPFO and 22,94±2,72 % by PPVO. Ash portions of carcasses represent about 14% of total body composition and hardly suffered any change since the start of the experiment. The highest value recorded was 14,15±1,21 % by FMVO; in the other hand the lowest was 13,36±1,01 % by PPVO. Fat and ash percentages were not affected by both protein and lipid sources. Energy of body carcasses was also similar between treatments and all were higher than the sample of the initial fishes (Figure 14). PPVO diet showed the highest value with 22,40±0,34 KJ/g while PPFO had the lowest value (21,30±0,66 KJ/g). It appears that the lipid source has a huge influence over the energy component of meagre, in a statistically significant manner ($P<0,05$) (Table 13). Phosphorus percentage was identical for all treatments with almost no difference from the initial sample. Phosphorus percentages varied between 2,18±0,22 by FMFO and 1,81±0,14 by PPFO. Statistical analysis over phosphorous percentage suggests it was only affected by the protein source.

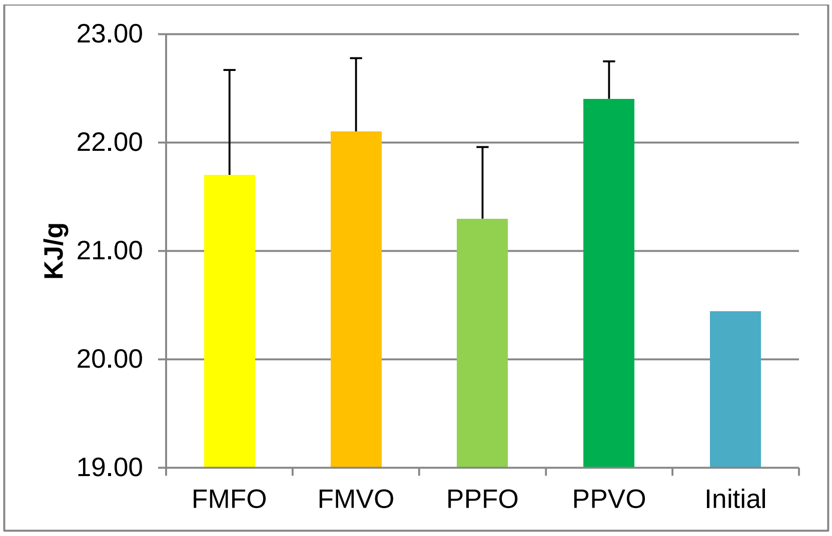

Fig. 14 – Energy quantity of body carcasses (KJ/g) of the treatments

Table 12 – Whole body composition (means and standart deviations)

Diet	DM %	Prot %	Fat %	Ash %	Ener KJ/g	Pho %
FMFO	27,56	61,90	21,32	13,82	21,70	2,03
	0,30	*2,30*	*0,91*	*0,79*	*0,97*	*0,25*
FMVO	27,91	59,90	20,88	14,15	22,10	2,18
	0,32	*1,01*	*1,70*	*1,21*	*0,68*	*0,22*
PPFO	28,87	56,21	19,52	13,37	21,30	1,81
	0,40	*2,03*	*1,15*	*1,07*	*0,66*	*0,14*
PPVO	28,29	60,04	22,94	13,36	22,40	1,89
	0,98	*1,20*	*2,72*	*1,01*	*0,34*	*0,06*

Table 13 – Statistical analysis of the whole body composition

	P Value	P Value	P Value
	Protein Source	Lipid Source	Interaction
DM	0,007*	0,742	0,03*
Prot	0*	0,234	0*
Fat	0,874	0,092	0,068
Ener	0,845	0,009*	0,193
Ash	0,099	0,57	0,57
Pho	0,009*	0,099	0,57

Values with * are statistically different (P <0.05)

4.4 Retention

Nutrient retention by meagre was quite good overall (Table 14). PPFO treatment had slightly higher percentage of protein intake, energy and dry matter than all others diets. PPVO allowed for highest percentage of fat intake while FMVO had a much higher percentage of phosphorus intake than any of other diets. Protein retention varied between 32,40±2,72 % (FMFO) and 36,04±2,75 % (PPFO) while fat intake varied between 27,50±1,16 % (FMVO) and 35,60±8,65 %. Phosphorus intake was lowest for FMFO with 43,81±9,89 % and highest for FMVO, with 58,54±10,53 %. Protein, fat, and phosphorus retention were not affected by either protein or lipid sources. The lowest value of energy belongs to the FMFO treatment with 24,67±2,20 while the highest was obtained by the PPFO dietary treatment with the 31,16±1,03. It appears energy retention was significantly affected by the protein source and also by the interaction between protein and lipid sources (P<0,05) (Table 15).

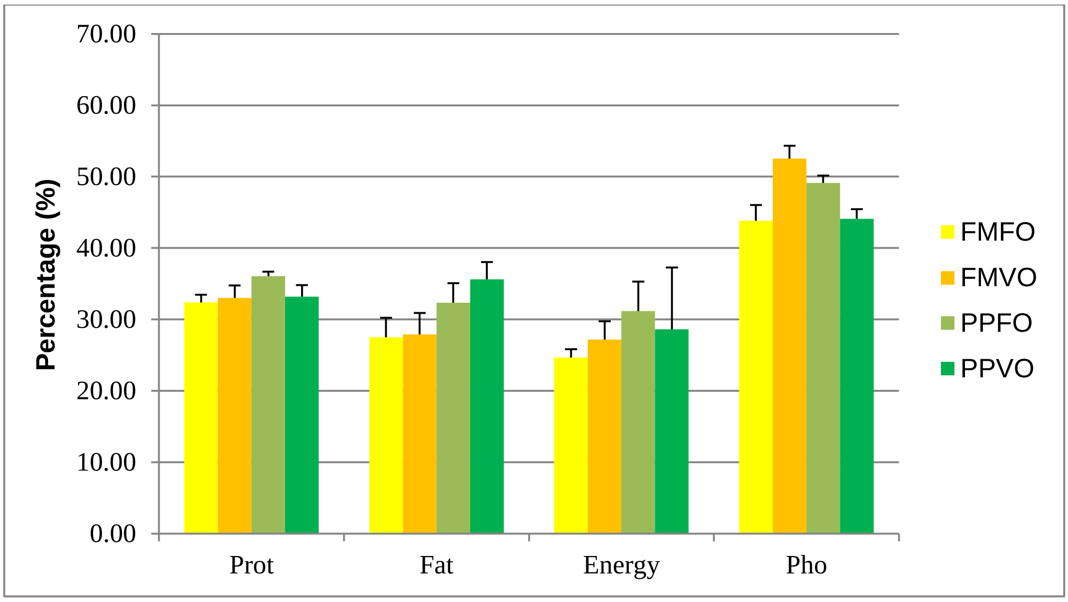

Fig. 15 – Retention (% intake) of the dietary treatments

Table 14 – Retention (% intake) (means and standart deviations)

Diet	DM	Prot	Fat	Ener	Pho
FMFO	24,62	32,40	27,50	24,67	43,81
	1,06	*2,72*	*1,16*	*2,20*	*9,89*
FMVO	26,46	33,01	27,90	27,19	52,54
	1,76	*3,01*	*2,54*	*1,81*	*10,53*
PPFO	32,90	36,04	32,32	31,16	49,14
	0,65	*2,75*	*4,15*	*1,03*	*6,95*
PPVO	26,70	33,18	35,60	28,60	44,08
	1,62	*2,43*	*8,65*	*1,37*	*2,67*

Table 15 – Statistical analysis of the retention (% intake)

	P Value Protein S	P Value Lipid S	P Value Inter
Prot	0,337	0,438	0,256
Fat	0,064	0,65	0,65
Ener	0,003*	0,881	0,031*
Pho	0,757	0,706	0,183

Values with * are statistically different (P <0.05)

5. Discussion

It is known that as we move towards the next decade, fishmeal and fish oil supplies are going to be more and more difficult to be available for the constant growing industry of aquaculture. According to FAO, fishmeal trade will remain positive for the year of 2013 as it still is an intense demand all over the world, despite its high price. The same source also predicts the price of fish oil will remain steady, after the small drop in the third quarter of 2012, which levelled off its price. Nevertheless the urgency of finding new alternatives for aquaculture sustainability is still a reality. Because of this, a big effort by the scientific community is being directed towards finding new ways to counter the lack of and the rise in price of these main products and this work appeared in this context.

5.1 Growth experiment

The rearing system proved to be well planned and built, as there were no reports of any kind of failure. The 12 tank system was well provisioned with the filtered water coming from the sea, which was important to maintain its chemical properties stable. Basically on this experiment, it was only necessary to control the percentage of oxygen in the water and its temperature, which turned out to simplify the work of keeping meagres juveniles of good health. On the colder weeks, it has been used gas to try and maintain water temperature over 16º C and despite in some days that turned to be impossible to do, meagres still kept on feeding, which was the main focus of the experiment.

Results of the growth experiment confirmed that meagre juveniles can grow pretty fast on the first stages of their life, which helps this specie to get a high potential for commercial aquaculture (Monfort, 2010). In 14 weeks of growth trial, meagre's weight nearly doubled for every treatment (FMFO: 115,9 g; FMVO: 115,2 g; PPFO: 121,2 g; PPVO: 109,5 g) which suggests these diets are near the ideal for fish feed needs. In fact, during the time of the experiment it was observed that juveniles did not show any signs of rejection of the diet given. With the manual feed of the juveniles, we understood why the behaviour of meagre while feeding can be described as being patient, but even on the days where the water temperature hit the lowest values, near 15 º C, they kept on feeding, despite the low quantity ingested. On the other hand, when the water temperature rose to its highest values, the behaviour of meagres towards feeding changed dramatically, to a more voracious behaviour, almost similar to the gilthead sea bream.

The strategy of manual feeding during day time (twice per day, one in the middle of the morning at 10:30 h and another by mid-afternoon at 15:30 h) plus the use of automated feeders during night time (that worked one time at 20:30 h) revealed to be acceptable. This way meagres were apparently fully satisfied since there were low numbers of fishes with injuries on their body and on their caudal fins.

As stated in the results, mortality was virtually negligible. We consider it this way due to the main mortality reason: most dead juveniles were found outside the tanks, having jumped, because of stress factors as quick changes in light conditions or induced vibrations in the tanks, for example, by accidental touch or other causes. Even the random bite between meagres could engage a chain reaction where all the fishes started a stressed behaviour with quick movements around the tanks, making some jump also. It was because of this that all tanks were properly covered by nets, in an attempt to reduce fish jumps to the outside; however, the more weight they gained throughout the experiment, the easier they could jump out, since their bigger size was sometimes enough to break the net.

5.2 Growth performance

At this point, we can say that in general terms the growth performance was not significantly affected by the source of the proteins or the lipids, after the statistical analysis by the two-way ANOVA. However, there were two points where the interaction between protein and lipid source seems to have some influence, which were the FCR and the PER indexes.

Beginning with the FBW, the best result achieved here was by PPFO diet (FBW=121,2±2,4 g), with bigger portion of vegetal protein included and provided with fish oil, which could generate some controversy as meagre is a carnivorous fish belonging to the 4.3 according to FishBase, and despite having a higher percentage of vegetal source, this treatment got the best result concerning final body weight. The control diet FMFO (which in theory would get the best results as it was based on the commercial diet used for the gilt-head sea bream) with an average of 115,9±4,8 g in the FBW, was lower than PPFO.

SGR levels for all treatments were similar but not significant considering our two sources of protein and/or fat. PPFO, following the result of the FBW, got the best result of all diets, with 0,89±0,02 %/day. Our results were slightly lower than other works with meagre, with values around 1,2 %/day for the best diets (Estévez *et al.*, 2011; Martínez-Llorens *et al.*, 2011) but similar with the values obtained by (Piccolo *et al.*, 2008) (around 0,90 %/day). It is known that SGR values can be directly influenced by the presence of methionine (Mai *et al.*, 2006; Zhou *et al.*, 2011), which is, like lysine, one of the most limiting amino acids to get in the raw materials of fish diets (Abimorad *et al.*, 2009; Sardar *et al.*, 2009). Adding DL-Methionine to the diets with high vegetal sources had for sure a crucial effect to get the best result by the PPFO diet. It is also known that water temperature can have a big impact on SGR values when food availability is not a limiting factor (Talbot, 1993) and considering that our trial was undertaken between September and December we can still consider these results as fairly acceptable.

Despite not having significance levels on FBW, the same does not happen when analysing the FCR index. In fact, our statistical analysis proved the interaction between protein and lipids source have a significant impact on the value of FCR as well as the protein source itself. All values of this index were quite good for the conditions of our experiment. Once more, PPFO got the best value, with the value of 0,99±0,04. To our knowledge, this is one of the lowest FCR indexes obtained by a dietary treatment on an experiment with meagre juveniles. Two works about meagre growth performance obtained similarly lower values, with the best diets achieving around 0,9±0,1 (Chatzifotis *et al.*, 2012; Mittakos *et al.*, 2012); the other treatments of our work ranged from 1,16±0,04 to 1,19±0,06 which can be compared to more similar and common results by other works: FCRs of around 1,2 (Martínez-Llorens *et al.*, 2011), or even higher than 1,2 (Velasco-Vargas *et al.*, 2013). This is a very important result as we could prove by this trial the viability of a partial replacement diet like PPFO, which can achieve excellent values without the need of high temperatures, as this experiment was undertaken with a mean temperature of 19,53±2,29 ºC.

In theory, VFI values are directly proportional and explained by the need of protein intake by the fishes. This statement can be supported by some works done in the past as they help to describe this relationship (Du *et al.*, 2005; Peres & Oliva-Teles, 1999; Santinha *et al.*, 1999). No significance was observed concerning VFI to the two different sources of proteins and oils, as in fact all percentages of VFI were pretty similar considering the various treatments. However, values of FM based diets were higher than the PP based, which can be analysed with some curiosity, as in theory probably we expect the inverse. Once more the balance of the dietary composition seems to work, although we cannot say they are significantly different, the vegetarian based meals showed to not be worse regarding the VFI percentages.

PER is one of the most important indexes to evaluate how the protein ingested by fish is treated (Gómez-Montes *et al.*, 2003). In this experiment, PER was greatly influenced by the interaction of protein and lipid source as well as by the protein source also. PPFO treatment got the highest value by far, when compared to the other diets, with 2,08±0,08 while the nearest result to this value was near 1,8. As we got four treatments that are isoenergetic and isoproteic, this high value of PPFO in the PER index can be explained by the final weight gain, that was in fact bigger than for any other treatment.

This work, appearing in a decade where there is an intense research effort in the improvement of partial replacement diets used in aquaculture, also helps to understand that it is now clear that plant-protein and oil sources are valid alternative ingredients for fish feeds (Gatlin *et al.*, 2007). The PPFO treatment had the best performance, since meagre juveniles gained more weight, when comparing to the other diets, in the same time. Having a big protein portion coming from plant derived feedstuff it is with some surprise we achieved this result. In the other hand, it is known that when provided essential amino acid requirements, the palatability of the feed is guaranteed and the levels of anti-nutritional factors are low, plant proteins can be successfully used in diets for marine fish species, such as gilthead seabream and European seabass (Benedito-Palos *et al.*, 2007; Dias *et al.*, 2009; Robaina *et al.*, 1995; Sánchez Lozano *et al.*, 2007; Tibaldi *et al.*, 2006). Recently it was proved that meagre has an enzymatic pattern well suited for protein digestion but a low capacity for digesting carbohydrates as in fact this is a carnivorous fish (Castro *et al.*, 2013), which in theory would indicate high protein based meals as main source of feedstuff to be used. However, after this work we can also say that meagre can achieve pretty decent growth performances when fed under well balanced partial replacement diets. We can also conclude the importance of the origin of proteins and lipids of the diets, as they seem to influence the FCR and the PER indexes.

5.3 Whole body composition and retention

Proximal composition of meagres rose in this trial were also analysed in the attempt to study the impact of the two different sources of protein and fat of the diets in body carcasses. There are some significant values regarding the two sources of protein and fat as they seemed to affect some values of the various parameters of meagre's whole body. Dry matter, protein, fat, energy, ash and phosphorus of the carcasses were evaluated and statistical analysis of these parameters suggest there are significant differences regarding protein and lipid interactions in dry matter and protein percentages. In addition, considering only the protein source, our data suggests that it seems to have influence in the percentages of the dry matter, protein and phosphorus. Concerning the lipid source, we also had significance in the energy values of the carcasses. While fat and ash quantity of the carcasses do not show any signs of being influenced by vegetal or marine sources, the same is not observed regarding dry matter, protein and phosphorus. Despite having similar values, dry matter percentages were slightly higher in diets provided with protein from vegetal sources (PPFO and PPVO), which caused our two way variance analysis to detect differences. Considering the protein percentages of the carcasses we can say that despite the values being very close between diets, there was some minor variation amongst them. Marine protein based diets (FMFO and FMVO) showed a slightly higher percentage on the protein content. If there is a real impact of the protein source of the diets it must be due to digestion induced alterations by vegetal nutrients. The fact that carnivorous fish require longer time to digest plant protein based diets (Buddington *et al.*, 1996; Santigosa *et al.*, 2008) may be one of the reasons to explain those differences, mainly due the higher content of ANFs of vegetal based diets, which can decrease enzymatic activity, forming complexes with minerals and proteins (Moyano López *et al.*, 1999; Santigosa *et al.*, 2008; Sugiura *et al.*, 1999). Phosphorus percentages were slightly higher in FMFO and FMVO, which made the significance regarding the two different sources. This could be related with the low capacity of digesting vegetal compounds with phosphorus by meagre as it is known that fishes have limited uptake for certain minerals provided with plant products (Naylor *et al.*, 2009). The only point where oils seem to have influence was the energy of the carcasses. Fishes fed with vegetal oil based diets (FMVO and PPVO) had slightly more quantity of energy than marine based meals which suggests a bigger efficiency regarding the provision of more energy over the conventional ones. In theory, this could also indicate a spare of proteins that could otherwise have been catabolized and used as an energy source (Chatzifotis *et al.*, 2010; De Silva *et al.*, 2001; Lee *et al.*, 2002; Skalli *et al.*, 2004).

Our results regarding whole body composition of meagre emphasizes that despite the differences between the dietary treatments there are no huge changes for the parameters evaluated, which was fairly expected. Initial body composition is almost identical to their final composition as well, suggesting little change in the dynamics of the chemical characteristics after the growth experiment under different diets.

As far as we know, this is the first work where proximal composition was evaluated concerning two different sources of protein and fat in partial replacement diets, which makes it difficult to compare to other works. However it is known that endogenous factors may have high influence in dry matter, ash content and protein levels of the carcasses as well as exogenous factors (like dietaries treatments) have a bigger impact in the lipid content of fish (Shearer, 1994). Our results contrast with the first part of this statement, as it seems that dry matter and protein percentages suffered variations depending on the diets. For the second part of the previous citation, having significance results on the energy values of the carcasses may emphasize the idea that exogenous factors are in the basis of the fat content, as a grand portion of energy is mainly given under the quantity and quality of lipids that the diets contain.

Retention values were similar between all dietary treatments as no big changes between their values were observed. Analysing retention values we concluded that there were significant differences in the energy retention concerning the protein source of the diets and, consequently, in the interaction of both sources. Vegetal based diets got higher values than the marine ones which explained statistical differences. This result seems to be in agreement with the idea of increased lipogenesis with fish meal replacement (Kaushik *et al.*, 2004), because of the higher energy retention values observed by PPFO and PPVO.

6. Conclusion

At the end of this experiment we can say that considering the partial replacement diets, meagre juveniles tolerated them with success. We determined that the source of protein and lipids has some impacts on the growth performance of the meagre: on the FCR and on the PER. Furthermore, the most surprising result resides in the fact the treatment PPFO, with higher percentage of plant protein and provided with fish oil, had the best result in terms of final body weight and FCR values. Also, we do not consider the performance of the other diets to be bad. Certainly, all of them had a worse performance when compared with the PPFO diet but they were viable considering their results. Having FMFO as the main comparison factor, FMVO and PPVO had a similar performance towards this control, with slight variations amongst the several growth parameters evaluated.

Whole body composition analysis permitted us to understand that the origin of protein and lipids of the diets can have influence over the dry matter, protein and phosphorus content of the carcasses. As for nutrient retention, no big differences were observed when comparing marine or plant origins, except for one point: it appears that the source of the diets have influence over the energy retention values. This is the first work to report such impacts on the proximal composition of meagre and its retention capacity, which can provide an important basis for further investigation about partial replacement diets.

This may prove the viability of using partial replacement meals with plant based ingredients, to be incorporated in diets for meagre. We believe that such success of these diets would also depend in the counter balance that was given to them, with special focus on adding concentrate of essential amino acids (L-lysine and DL-methionine) in high percentages diets with plant origins (PPFO, PPVO), which for sure had an important role to provide an healthy and efficient growth of the juveniles. For next trials, aside from improving meagre's experimental dietary treatments, it would be interesting to study the flesh quality of reared meagre fed with similar partial replacement diets, to evaluate potential commercial purposes for human consumption. For sure, in the next years we will assist to new findings concerning new balanced diets for meagre, since there is still no specialized diet for the best grow of this species.

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